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Original Article

Bioaccumulation of Some Heavy Metals in the Liver Flukes *Fasciola hepatica* and *F. gigantica*

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Abstract

Background: Heavy metals tend to bioaccumulate in living organisms, and their accumulation has been a major concern. As mammals are known to excrete heavy metals via their bile, it seems to be very promising to analyse metal burdens of parasites that infect the biliary tree such as liver flukes of the genus *Fasciola*. The present study was carried out to evaluate *F. hepatica* and *F. gigantica* as bioaccumulators of heavy metals, and to estimate their use as sensitive markers of environmental pollution with heavy metals.

Methods: A total of 36 slaughtered buffaloes (26 infected and 10 controls) collected from the slaughter-house of Tanta City, Egypt were used. Samples of muscle and liver tissues were taken from each buffalo. A total of 44 adult *Fasciola* flukes were collected from the 26 infected buffaloes. Quantification of some heavy metals (Cd, Cr, Cu, Pb and Zn) in samples was carried out using electrothermal atomic absorption spectrometry.

Results: Results revealed different concentrations of heavy metals in different host tissues. The adult flukes were classified into *F. hepatica* (n = 25) and *F. gigantica* (n = 19). The bio-concentration factor (BCF) of Cr was significantly higher in *F. hepatica* ($P = 0.0465$) while BCF of Zn was significantly higher in *F. gigantica* ($P = 0.0189$). A comparative study between the two species as regards the BCF was never done before.

Conclusion: The obtained results indicate the possibility of use of *Fasciola* flukes as markers of environmental pollution with some heavy metals.

Introduction

Heavy metals can be defined as elements which have atomic weight between 63.546 and 200.590 (1), and are characterized by a specific gravity greater than 4.0 (2). They are unavoidable components of our environment. Heavy metals are present in varying quantities throughout the geosphere and are cycled continuously through different components of the ecosphere. The amounts of different heavy metals in ambient atmospheres have been increasing with advances of human civilization and are likely to increase further with increasing exploitation of geological resources, such as mining and fossil fuel development (3, 4). Although minute quantities of certain heavy metals are essential for some biological systems like zinc, copper and chromium, and others may provide beneficial effects in animals like selenium and vanadium, their excesses often are associated with harmful effects in man and animals (5-7). Heavy metal toxicity may cause significant health problems, such as retarded growth and development, cancer, organ damage, nervous system damage, and sometimes death (8).

The toxicity of metals known to be wholly harmful like cadmium and lead rises with their concentration in an accessible form especially by organisms in food chains. Since the beginning of the twentieth century the concentrations of heavy metals in soil and water have dramatically increased due to increased human activities. Urban areas that have metal-processing activities are often highly polluted with heavy metals than rural areas (5). Also, heavy metal concentrations of agricultural soils have reached critical levels in some parts of the world. Because of heavy metal contamination, some agricultural villages in Eastern Europe have been deserted, and in Japan, nearly 10% of rice paddy lands can no longer be used (9).

Bio-monitoring of heavy metals is an important rapidly growing field that uses several organisms as bio-indicators (10). Fish parasites have been identified as highly sensitive to environmental pollution either because of their physiological response to contaminants or because of their ability to accumulate certain toxic substances (11, 12). Some intestinal parasites have the ability to accumulate heavy metals at concentrations that are many times higher than those recorded in the host tissues (11). Acanthocephalans are the group most frequently used in aquatic ecotoxicological studies (11, 13-15). Representatives of other groups of helminths rarely appear in such studies (16-19).

Humans and other mammals eliminate part of heavy metals from the body via the bile which is secreted into the duodenum and eliminated with faeces (20). Thus, it may be relevant to study the bioaccumulation of heavy metals by the parasites that inhabit the biliary tree of the mammalian host like liver flukes of the genus *Fasciola*. This genus was one of the first pathogens to be discovered and incriminated in causing infectious diseases (21). *Fasciola* parasites have a cosmopolitan distribution, and fascioliasis is not uncommon in many countries of the world (22, 23).

The main objective of the present study was to evaluate *F. hepatica* and *F. gigantica* as bioaccumulators of heavy metals, and to estimate their use as sensitive markers of environmental pollution with heavy metals. This objective was achieved through determination of the concentrations of the heavy metals Cd, Cr, Cu, Pb and Zn in the two species, in comparison to those in the definitive host tissues (liver and muscles).

Materials and Methods

Sample collection and identification

A total of 44 adult *Fasciola* flukes were collected from the 26 infected buffaloes slaughtered at the Abattoir of Tanta City. The flukes were washed in physiological saline, identified

and classified into *F. hepatica* and *F. gigantica* according to the parameters described by Lotfy and his colleagues in 2002 (24). Samples of the host liver and muscles were collected from the infected buffaloes and from 10 uninfected animals as a control group. All the samples of flukes and host tissues were frozen for up to 4 weeks at -26 ºC prior to processing for analysis of heavy metals.

Sample preparation and quantification of the metallic contents

All fluke and tissue samples were digested according to the method described by Mascia and his colleagues in 1990 (25). Quantification of the metallic (Cd, Cr, Cu, Pb and Zn) contents of the digested samples and blanks was carried out using UNICAM 969 Atomic Absorption Spectrometer with flame atomization (Thermo Elemental, UK) at Medical Technology Centre - Extension of Medical Research Institute. The Spectrometer was pre-calibrated using certified standards. The bio-concentration factor of the metal in the parasite tissue was calculated according to the formula (16):

$$\text{The bio-concentration factor} = \frac{\text{Conc. of the heavy metal in the parasite tissue}}{\text{Conc. of the heavy metal in the host liver}}$$

Statistical analyses

Statistical analyses were run on an IBM compatible PC using GraphPad Prism 5 for windows statistical package (Version 5.01; GraphPad Inc., La Jolla, CA). All parameters were treated as non-parametric data. They are expressed as median±SEM (min-max), whereas SEM means the standard error of the mean. Wilcoxon signed rank test was used to compare paired data. Mann-Whitney test was used to compare unpaired data. The *P* value below 0.05 was considered significant.

Results

Fasciola flukes used in the present study were identified and classified into 25 *F. hepatica* and 19 *F. gigantica*. In healthy buffaloes, by comparing the concentrations of metals in liver tissues and muscles (Table 1), Cu showed significantly higher concentration in liver tissues compared to muscles ($P = 0.0020$). On contrary, the level of Zn was significantly lower in liver tissues ($P = 0.0059$). In infected buffaloes, by comparing the concentrations of metals in liver tissues and muscles (Table 2), Cu showed significantly higher concentration in liver tissues compared to muscles ($P < 0.0001$), while the levels of Cr and Zn were significantly lower in liver tissues ($P = 0.0214$ and 0.0021 respectively). By comparing the concentrations of heavy metals in liver tissues of infected and uninfected buffaloes (Table 3), only Pb and Zn showed significant differences between the two groups of animals ($P = 0.0179$ and 0.0349, respectively). On the other hand, by comparing the concentrations of heavy metals in muscles of infected and uninfected buffaloes (Table 4), all metals were higher in the infected group. Four metals (Cd, Cr, Cu and Pb) showed significant differences between the two groups of animals ($P = 0.0403$, 0.0340, 0.0042 and 0.0024, respectively).

Generally, the levels of heavy metals in the host liver tissues were lower than those in *Fasciola* flukes. By comparing the concentrations of heavy metals in *F. hepatica* and the definitive host liver tissues (Table 5), only Cr, Cu and Pb showed significant differences ($P = 0.0064$, < 0.0001 and < 0.0001, respectively). On the other hand, by comparing the concentrations of heavy metals in *F. gigantica* and the definitive host liver tissues (Table 6), Cd, Cu, Pb and Zn showed significant differences ($P = 0.0347$, 0.0024, 0.0004 and 0.0255 , respectively). The bioaccumulation of the metals in *F. hepatica* and *F. gigantica* was studied by calculating BCF for each metal. By comparing the levels of the BCF in the two species (Table 7), the BCF of Cr was significantly higher in *F. hepatica* ($P = 0.0465$) and the BCF of Zn was significantly higher in *F. gigantica* ($P = 0.0189$).

Table 1: Concentrations of heavy metals in liver tissues and muscles of healthy buffaloes

Heavy metals	Animal tissues (n = 10)		Wilcoxon signed rank test (*P* value)
*	Liver	Muscles	
Cd	0.31±0.04(0.13-0.49)	0.26±0.04(0.13-0.54)	0.6101
Cr	3.67±0.20(2.45-4.45)	3.56±0.22(2.23-4.67)	1.0000
Cu	11.32±1.01(8.02-16.05)	4.01±0.25(2.34-5.23)	0.0020
Pb	5.14±0.64(2.40-8.11)	3.94±0.73(2.17-8.56)	0.4258
Zn	49.07±3.75(33.90-76.10)	66.30±4.94(43.75-95.25)	0.0059

*Concentrations of metals expressed as µg/g wet weight of tissue

Table 2: Concentrations of heavy metals in liver tissues and muscles of infected buffaloes

Heavy metals*	Animal tissues (n = 26)		Wilcoxon signed rank test (*P* value)
	Liver	Muscles	
Cd	0.36 ± 0.033(0.12-0.66)	0.36±0.029(0.15-0.70)	0.7425
Cr	3.60 ± 0.10(2.82-4.72)	4.30±0.24(2.00-6.41)	0.0214
Cu	13.18 ± 1.29(2.44-25.88)	5.31±0.33(1.95-10.74)	< 0.0001
Pb	7.08 ± 1.59(2.97-33.25)	10.07±1.12(3.20-25.56)	0.9899
Zn	77.13±5.22(27.46-147.40)	86.15±5.26(36.85-123.15)	0.0021

*Concentrations of metals expressed as µg/g wet weight of tissue

Table 3: Concentrations of heavy metals in liver tissues of infected and healthy buffaloes

Heavy metals*	Infected host liver (n = 26)	Uninfected liver (n = 10)	Mann-Whitney test (*P* value)
Cd	0.36±0.033(0.12-0.66)	0.31±0.04(0.13-0.49)	0.1574
Cr	3.60±0.10(2.82-4.72)	3.67±0.20(2.45-4.45)	0.4797
Cu	13.18±1.29(2.44-25.88)	11.32±1.01(8.02-16.05)	0.2367
Pb	7.08±1.59(2.97-33.25)	5.14±0.64(2.40-8.11)	0.0179
Zn	77.13±5.22(27.46-147.40)	49.07±3.75(33.90-76.10)	0.0349

*Concentrations of metals expressed as µg/g wet weight of tissue

Table 4: Concentrations of heavy metals in muscles of infected and healthy buffaloes

Heavy metals*	Infected host muscles (n = 26)	Uninfected muscles (n = 10)	Mann-Whitney test (*P* value)
Cd	0.36 ± 0.029 (0.15-0.70)	0.26 ± 0.04 (0.13-0.54)	0.0403
Cr	4.30 ± 0.24 (2.00-6.41)	3.56 ± 0.22 (2.23-4.67)	0.0340
Cu	5.31 ± 0.33 (1.95-10.74)	4.01 ± 0.25 (2.34-5.23)	0.0042
Pb	10.07 ± 1.12 (3.20-25.56)	3.94 ± 0.73 (2.17-8.56)	0.0024
Zn	86.15±5.26 (36.85-123.15)	66.30±4.94 (43.75-95.25)	0.1715

*Concentrations of metals expressed as µg/g wet weight of tissue

Table 5: Concentrations of heavy metals in *F. hepatica* and the definitive host liver tissues

Heavy metal*	*F. hepatica* (n = 25)	Liver tissues (n = 25)	Wilcoxon signed rank test (*P* value)
Cd	0.38±0.033(0.14-0.76)	0.36±0.03(0.12-0.65)	0.9886
Cr	4.15±0.25(2.44-7.56)	3.50± 0.11(2.82-4.72)	0.0064
Cu	19.32±3.96(6.35-76.39)	13.67±1.33(2.44-25.88)	< 0.0001
Pb	20.21±1.92(9.59-43.75)	6.74±1.66(2.97-33.25)	< 0.0001
Zn	71.95±4.76(32.77-112.30)	81.90±5.39(27.46-147.40)	0.9357

*Concentrations of metals expressed as µg/g wet weight of tissue

Table 6: Concentrations of heavy metals in *F. gigantica* and the definitive host liver tissues

Heavy metal*	*F. gigantica* (n = 19)	Liver tissues (n = 19)	Wilcoxon signed rank test (*P* value)
Cd	0.31±0.03(0.16-0.58)	0.36±0.04(0.19-0.66)	0.0347
Cr	3.46±0.33(2.12-7.62)	3.50±0.12(2.82-4.64)	0.8880
Cu	24.91±4.4(11.21-65.68)	15.22±1.34(6.59-25.88)	0.0024
Pb	23.97±5.05(10.96-88.31)	6.74±1.91(2.97-33.25)	0.0004
Zn	83.20±9.46(25.54-173.70)	83.21±6.30(27.46-147.40)	0.0255

**Concentrations of metals expressed as μg/g wet weight of tissue*

Table 7: The bio-concentration factor (BCF) for each of *F. hepatica* and *F. gigantica*

Heavy metal	*F. hepatica* BCF (n = 25)	*F. gigantica* BCF (n = 19)	Mann-Whitney test (*P* value)
Cd	0.98±0.17(0.33-3.83)	0.85±0.08(0.44-2.03)	0.1354
Cr	1.10±0.08(0.87-2.30)	0.95±0.09(0.49-1.81)	0.0465
Cu	1.83±0.23(0.37-6.20)	1.45±0.27(0.84-5.11)	0.2554
Pb	2.54±0.26(0.85-5.57)	2.62±0.56(0.80-8.92)	0.4626
Zn	0.94±0.05(0.49-1.56)	1.26±0.10(0.83-2.09)	0.0189

Discussion

The present study revealed different concentrations of heavy metals in different tissues of the studied buffaloes. By comparing the concentrations of heavy metals in liver tissues and muscles of healthy or infected buffaloes (Tables 1 & 2), Cu showed significantly higher concentration in liver tissues compared to muscles, but Zn showed significantly lower concentration in liver tissues. Only in infected buffaloes, the concentration of Cr was significantly lower in liver tissues compared to muscles (Table 2). The present results may be supported by the findings reported by others. In 2001, Abou-Arab studied the heavy metal contents in tissues of buffaloes from different areas in Egypt. According to his results Cd, Cu and Pb showed higher levels in liver tissues compared with muscles, but Zn showed higher levels in muscles compared with liver tissues (26). In 2004, Gasparik and his colleagues studied the concentration of some metals in liver tissues and muscles of the red deer, *Cervus elaphus*. The concentration of Cr was very similar in all the tissues they studied. The level of Cu was significantly higher in liver tissues, but the concentrations of Pb and Zn were significantly higher in muscles (27).

By comparing the concentrations of heavy metals in liver tissues of infected and uninfected buffaloes, only Pb and Zn were significantly higher in liver tissues of the infected group (Table 3). While, all metals were significantly higher in muscles of the infected group (Table 4). *Fasciola* spp. and other trematodes which utilize the liver as the main site of infection have been found to impair hepatic xenobiotic-metabolizing activity (28, 29). Thus, it could be concluded that liver damage by fluke infection may lead to accumulation of heavy metals in the host tissues which explains our present results. The adverse effects of the high concentrations of heavy metals in the host body on the immune system cannot be ignored. In 1993, Benkova and his colleagues showed that industrial emissions of Cr, Cu, Pb, and Zn decreased the phagocytic activity, the phagocytic index and complement levels in lambs, experimentally infected by *F. hepatica* (30). However, it is to be noted here that some metals such as Cu, Zn, Mn and Fe are co-factors for many enzymes and play an important role in many physiological functions of man and animals. Deficiency of these metals causes

disturbances and pathological conditions (31, 32). By comparing the concentrations of heavy metals in liver flukes and the host liver tissues, Cr, Cu and Pb were significantly higher in *F. hepatica* compared with the host liver tissues (Table 5). While, Cd, Cu, Pb and Zn were significantly higher in *F. gigantica* compared with the host liver tissues (Table 6). These present results may indicate the possibility of use of *Fasciola* flukes as markers of environmental pollution with some heavy metals. In 1998, Sures and his colleagues studied the accumulation of Pb and Cd in *F. hepatica*. They reported that *F. hepatica* accumulated Pb to a concentration considerably higher than that in the tissues of naturally infected cattle, but the Cd level was, by contrast, only approximately as high as that of the host's muscle and below its level in the liver tissue (16). The difference between our results and the results of Sures and his colleagues in 1998 could be attributed to the relatively small sample size (non-parametric data) in the two studies. In the study of Sures and his colleagues the sample size was 17 flukes (16).

By comparing the levels of the BCF in the two species, the BCF of Cr was significantly higher in *F. hepatica*, while the BCF of Zn was significantly higher in *F. gigantica*. Sures and his colleagues studied the BCF of Pb and Cd in *F. hepatica*. They reported that it was ranging from 4 to 80 for Pb and 0.1 to 3 for Cd (16). A comparative study between the two species as regards the BCF was never done before. Thus, the present BCF results should be considered preliminary and need to be confirmed by further studies.

Conclusion

The results of the present study indicate that *Fasciola* flukes are useful markers of environmental pollution with some heavy metals. Also, the present results suggest usage of flukes in assessment of the environmental deterioration by such metals.

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